HYPOCALCEMIA IN NEONATES WITH HYPOXIC ISCHEMIC ENCEPHALOPATHY: A CROSS SECTIONAL STUDY

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ABSTRACT

Objectives: *To determine the frequency of hypocalcemia in neonates with hypoxic-ischemic encephalopathy (HIE) and explore some of the associated factors.*

Methods and materials: *A cross-sectional study conducted at Lady Reading Hospital (LRH) Peshawar, Khyber Pakhtunkhwa, from January-June 2019. The sample size was 116 using 18% proportion of patients with HIE in the nursery, 95% confidence level and 07% margin of error, under the WHO sample size calculator. We employed consecutive non-probability sampling and included neonates who presented to the neonatal unit of LRH with more than one-minute delayed cry, 1-minute APGAR score of 3 or less, and neurological impairment evaluated for hypocalcemia. The parents/caregivers consented, once the purpose and benefits of study were explained to them. For serum calcium (total ionized), a 5ml venous blood sample was taken from all included neonates without applying the tourniquet to avoid artefactual hemoconcentration.*

Results: *There were total 116 neonates with HIE. Among these 76 (65.5%) were males while the mean age of the sample was 1.9 + 0.8 days. Hypocalcemia was recorded in more than a quarter (33.6%) of neonates. The occurrence of hypocalcemia was significantly different among the categories of age of neonate at birth, weight at birth, and educational status of mothers.*

Conclusion: *Hypocalcemia was common in neonates who presented with HIE. However, for more conclusive evidence the association between serum calcium and HIE needs to be studied in details particularly case control studies for better understanding of effect modifier and risk factors associated.*

Key words: *Neonates, Hypoxic Ischemic Encephalopathy, Hypocalcemia*

INTRODUCTION

Among every 1000 live births, 2-5 neonates suffer from neonatal encephalopathy, which is a primary cause of infant mortality and long-term illness. Hypoxemia, according to Volpe, is the decrease in the oxygenated blood flow, while cerebral ischemia is the "reduced quantity of blood to perfuse the brain.". The two types of oxygen deprivation are important, but cerebral ischemia is more significant because it also causes glucose deprivation, which plays role in the development of neuronal damage[1]. The global issue of term and near-term infants with hypoxic ischemic encephalopathy (HIE), also known as hypoxic ischemic brain damage, has persisted. Hypoxic ischemic encephalopathy is the primary cause of encephalopathy in the newborns and despite substantial advancements in prenatal care over the past few decades, it is still regarded as a serious illness that can cause significant death and illness[2,3]. Clinical and biochemical proof of acute or subacute

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brain damage caused by asphyxia, also known as HIE or neonatal asphyxia or hypoxia, is what distinguishes it from other types of asphyxia. After early stabilization and restoration of vitals, HIE therapy is mostly supportive and should concentrate the following factors: sufficient breathing, managing fluids, avoiding hypo and hyperglycemia, treating seizures, preventing hyperthermia, adequate perfusion, and blood pressure control. Moreover, studies show that for infants with moderate to severe HIE, therapeutic hypothermia (33º-33.5ºC for 72h) followed by slow and controlled rewarming is necessary, along with maintaining a mean blood pressure (BP) above 35-40 mm Hg to prevent decreased cerebral perfusion[4,5]. Systemic hypoxia and/or diminished cerebral blood flow (CBF) are the main contributors of HIE.

The perinatal hypoxia causes almost 23% of neonatal deaths, around the globe[6]. Presently, the majority of the care given to a baby with encephalopathy focused on supportive measures to improve and maintain cerebral perfusion, maintain appropriate gas exchange, and treat seizures[7-9]. In addition, hypocalcaemia in newborns under 3 days is known to be caused by perinatal hypoxia. Neuromuscular irritability brought on by hypocalcemia may manifest as convulsions, jitteriness, twitching, and/or irritation. It might also lead to general symptoms including poor eating routines, sluggishness, and vomiting. Infants who have asphyxiated frequently suffer from hypocalcemia. Although hyperthermia is known to be linked with increased hazardous outcomes in newborns with moderate to severe HIE, therapeutic hypothermia is still regarded as a standard of therapy for deteriorating levels of HIE[10]. By reducing calcium (Ca) influx into the cells during the reperfusion period, hypothermia enhances neuroprotection. Therapeutic hyperthermia can raise serum Ca levels and may potentially counteract the effects of HIE's hypocalcemia[11].

By inhibiting intracellular calcium influx, which may raise serum calcium levels and maintain homeostasis, therapeutic hypothermia supports neuroprotection through a number of methods[12]. As a treatment for newborns who have asphyxiated, induced hypothermia shows promising results in lowering neurodevelopmental impairments in those who survive[13]. Convulsion was the most prevalent symptom among asphyxiated newborns with early-onset hypocalcemia, according to a study done in Nigeria that found an overall frequency of 22.6 percent hypocalcemia in term neonates with prenatal asphyxia[14,15]. In our setup, perinatal asphyxia is a commonly observed phenomenon and is linked to a high fatality rate. There is a dearth of local research to establish the prevalence of hypocalcemia in newborns with asphyxia, few studies have indicated that it occurs in neonates with asphyxia though. Knowing the actual cause of an asphyxiated neonate's condition is also essential in order to start the appropriate therapy, halt the subsequent difficulties, and assist medical professionals in making decisions about how to care neonates with prenatal asphyxia. Therefore, we aim to find out how frequently hypocalcemia occurs in newborns with hypoxic ischemic encephalopathy and also look at some of the associated factors.

MATERIALS AND METHODS

We conducted a cross-sectional study between January to June 2019 at the pediatric department of Lady Reading Hospital (LRH) Peshawar, Khyber Pakhtunkhwa. LRH is the largest public sector tertiary care hospital of the province. The Sample size was 116 using 18% proportion of patients with hypoxic ischemic encephalopathy in nursery[13], 95% confidence level and 07% margin of error, by using sample size calculator of WHO. We employed consecutive non-probability sampling and included all neonates (both male and female) from birth to three days of age. Based on history or medical records, to avoid any confounding effects or potential introduction of bias to the study, we excluded: premature neonates, neonates with tracheo-esophageal atresia, and other congenital deformities like micro or macrocephaly or hydrocephaly.

The study was approved by the ethics board of Khyber medical university (Dir/KMU-EB/HN/000120) and ethical review board of MTI-LRH (241/LRH/MTI). All neonates presented to the neonatal unit of LRH with more than one-minute delayed cry, 1-minute APGAR score of 3 or less, and neurological impairment evaluated for hypocalcemia, were included in this study. Normally the ionized serum Ca level for term infant over the first 72 hours of life are 1.22 to 1.24 mmol/L (4.88-4.96 mg/dl). Ionized calcium level of <4mg/dl was considered as hypocalcemia in this study. The parents/caregivers consented after getting an explanation about the purpose and benefits of the study. For serum calci-

um (total ionized), a 5ml venous blood sample was taken from all included neonates without applying the tourniquet to avoid artefactual hemo-concentration. Total calcium was determined by Cobas Roche analyzer and ionized calcium was determined by using a machine called easylyte. Each sample was processed within 24 hours of collection. Infant with hypocalcemia was treated with intravenous calcium gluconate, where no infant was treated before the serum calcium level measurement.

All the data collected on a predesigned proforma was analyzed through SPSS 22. We calculated frequencies and proportions for categorical variables like hypocalcemia, age (categorized), gestational age (categorized) and weight of the neonate (categorized). Mean and standard deviation (SD) was calculated for age. Post stratification chi square test was applied and association of hypocalcemia was assessed with variables like: age, weight, gender, gestational age and mother's educational status. P-value ≤0.05 was taken as significant. The main findings are presented in tables.

RESULT

There were total 116 neonates with hypoxic ischemic encephalopathy (HIE). Among these 76 (65.5%) were males while the mean age of the sample was 1.9 + 0.8 days. We divided the age in 3 different groups i.e., age group of 1-day, more than 1 to 2 days, and more than 2 to 3 days, where the first group was leading with around 40% proportion (table 1). Mean gestational age at birth was 38.8 + 3.2 weeks. We categorized the gestational age as pre-term (< 37 weeks), term (37 to 42 weeks) and post-term (> 42 weeks), where more than half of the sample was term neonates (table 1). Mean weight of the neonate at birth according to the records was 2.96 + 0.5 kg. Table 1 shows three categories of neonates' weights: underweight (< 2.5kg), normal weight (2.5 to 3.5kg) and overweight (> 3.5kg), where more than 60% neonates with HIE were reported in normal weight group. Mothers of most of the neonates included in the study were either illiterate or having literacy of up to matric (table 1). The mean serum ionized calcium level was 4.2 + 0.8mg/dl. According to the operational definition of hypocalcemia, it was recorded in more than a quarter (33.6%) of neonates (table 2). The last table elaborates the significance of difference in hypocalcemia among the different categories of variables including: age at birth, gender of neonate, gestational age at birth, weight at birth and educational status of mothers. Among these variables the occurrence of hypocalcemia was significantly different among the categories of age of neonate at birth, weight at birth, and educational status of mothers at p-value of <0.05 (table 3).

DISCUSSION

For newborns with hypoxic ischemic encephalopathy, hypocalcemia is a serious issue. The purpose of this study was to ascertain the frequency of hypocalcemia in newborns with hypoxic ischemic encephalopathy and any contributing variables. The main findings of this study shows that more than 30% of the neonates with HIE were suffering from hypocalcemia. Among the neonates who were reported to Leady Reading hospital with HIE and enrolled

Table 1: Demographic characteristics of neonates with HIE (N=116)

Characteristic	n	%
Gender of neonates with HIE		
Male	76	65.5
Female	40	34.5
Age of neonates with HIE (days)		
1 day	46	39.7
> 1 to 2 days	37	31.9
> 2 to 3 days	33	28.4
Gestational age categories of neonates with HIE		
Preterm	34	29.3
Term	65	56.0
Post-term	17	14.7
Birth weight categories of neonates with HIE		
Underweight	28	24.1
Normal weight	70	60.3
Overweight	18	15.5
Educational status of the mothers		
Illiterate	44	37.9
Middle	22	19.0
Matric	35	30.2
Intermediate or higher	15	12.9

Table 2: Hypocalcemia in neonates with HIE

Hypocalcemia	n	%
Yes	39	33.6
No	77	66.4
Total	116	100.0

Table 3: Association of Hypocalcemia with different variables

Variables	Hypocalcemia		
Age of neonate with HIE (days)	Yes	No	P-Value
1 day	22 (47.8%)	24 (52.2%)	0.032
> 1 to 2 days	9 (24.3%)	28 (75.7%)	
> 2 to 3 days	8 (24.2%)	25 (75.8%)	
Gender of neonates with HIE			
Male	30 (39.5%)	46 (60.5%)	0.066
Female	9 (22.5%)	31 (77.5%)	
Gestational age of neonates with HIE			
Pre-term	13 (38.2%)	21 (61.8%)	0.256
Term	18 (27.7%)	47 (72.3%)	
Post-term	8 (47.1%)	9 (52.9%)	
Weight at birth of neonates with HIE			
Underweight	7 (25%)	21 (75%)	0.001
Normal weight	32 (45.7%)	38 (54.3%)	
Overweight	0 (0.0%)	18 (100%)	
Educational status of the mother			
illiterate	25 (56.8%)	19 (43.2%)	< 0.001
Middle	0 (0.0%)	22 (100%)	
Metric	8 (22.9%)	27 (77.1%)	
Intermediate or higher	6 (40%)	9 (60%)	

in this study; the occurrence of hypocalcemia was significantly associated with weight of neonates, age of neonates, and educational status of the mothers.

The mean gestational age at birth was 38.8 + 3.2 weeks in the current study which was close to the gestational age of 39 weeks reported in a similar study conducted at Kenya[14]. Hypocalcemia, in our study was reported in 33.6% of the neonates. An Indian study reported hypocalcemia to be present among neonates with asphyxia or HIE[16]. In India, perinatal hypoxia is the most frequent main cause of neonatal death (28.8%) and morbidity, as well as the leading cause of stillbirths (45.1 percent)[17]. According to a research conducted in Nigeria, the prevalence of hypocalcemia among newborns with severe HIE was 22.6 percent, which is lower than the results of the current study[18]. The results of our study are close to those of a study carried out in 2016, which found that 31.6 percent of normal term infants had neonatal hypocalcemia[19].

Based on the findings of our study, the hypocalcemia in neonates with HIE was significantly associated with birth weight of the neonates ($p<0.001$). These findings were supported by the literature that lower birthweights of neonates results in hypocalcemia[20]. The three main electrolytes in a person's body are sodium, potassium, and calcium; any variation in normal blood levels of these electrolytes can result in convulsions, shock, and other metabolic disorders. In addition to aiding in the function of muscles and serving as a cofactor for numerous enzymes related processes, calcium is a crucial second messenger in our bodies[21]. There is ongoing discussion over the reasons for hypocalcemia in cases of birth asphyxia[22]. Hypocalcemia can result from a number of factors, including perinatal asphyxia, delayed feed introduction, increased calcitonin synthesis, increased endogenous phosphate load, renal insufficiency, and decreased secretion of parathyroid hormone[20].

Considering the sample size of current study, it was close to that of another similar study, which reported 21% hypocalcemia in non-therapeutic hypothermia group neonates with HIE[12]. However, our study domain did not include TH to be studied on the included sample. Similar findings were reported by another similar study reporting lower levels of calcium in pre-TH neonates with HIE[13].

The first three days of life were considered in our study to evaluate calcium levels. Lowe et al. showed that the mean total and ionized blood calcium concentration was considerably lower in HIE, different finding from many studies including big RCTs. Albumin level was also low in this study[23]. Moreover, Lowe et al. discovered low calcium levels between 60 and 72 hours; however, our values, which were conducted mostly earlier than this in the first three days, may be the cause of the discrepancy in data. Thus, the values might be compared if total calcium, albumin adjusted calcium, and ionized calcium were measured in major prospective studies[23]. When TH was present, hypocalcemia was more prevalent; this might be a neuroprotective mechanism that prevents calcium from entering the cells.

Hypocalcemia in HIE neonates is also believed to be associated with different maternal factors including the intake of calcium supplementation during pregnancy, mode of delivery, and the facility used for delivery. However, an in-depth exploration of these factors in future studies can help understand the as-

sociation of these factors in hypocalcemia and HIE.

CONCLUSION

Hypoxic ischemic encephalopathy in neonates is a significant reason for hypocalcemia and the consequent associated complications in these neonates. Hypocalcemia was significantly associated with birthweight of the neonates also the educational status of the mothers, the later finding needs further exploration to know the relation between education level of mothers and occurrence of hypocalcemia in their neonates.

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